Available online at https://www.ijasrd.org/

International Journal of Advanced Scientific Research & Development

Vol. 05, Iss. 05, Ver. I, May' 2018, pp. 01 – 12

e-ISSN: 2395-6089
p-ISSN: 2394-8906

IMMUNOMODULATION AND ANTIHYPERTENSITY STUDY OF GREEN TEA EXTRACTS INDUCED WITH HYDROCORTISONE BY USING ANIMAL MODEL

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ARTICLE INFO

Article History:

Received: 07 Apr 2018;
Received in revised form:
16 May 2018;
Accepted: 16 May 2018;
Published online: 10 Jun 2018.

Key words:

Immunomodulation,
Green Tea,
Epigallo Catechin,
Hydrocortisone.

ABSTRACT

Green tea and their constitutents play an important role in defense against pathogens to the modulation of immune system. Antioxidant and other substances in green tea helps to protect the heart and blood vessels. Antimicobial activity of green tea extract was determined by disc diffusion method the results shown the maximum zone of inhibition of green tea extract for P. aeruginosa. While the inhibition zone with 13mm was observed with P. fluorescence followed by E. coli and Shigella flexneri. The present study green tea extract was given to mice for four weeks to assess the immune response by measuring phagocytic cells and antibody production. Administration of EGTE 200 mg/kg exhibited 2.76 ± 0.22. The result revealed EGTE from green tea enhance multiplication of leukocytes. Studies related to polymorphic cells observed as differencial count expressed 42% cells/cubic mm meadiated with hydrocortisone alone. The lymphocytes was found to be 58% cells/cubic mm enhanced with green tea and hydrocortisone combination. The effect of serum biomarkers SGOT and SGPT observed for 15 days with oral administraion of green tea revealed body weight was decreased after the intake of green tea. Both SGOT and SGPT was found to increase. A remarkable difference was observed with the marker enzyme SGPT when hydrocotisone treated mice revealed 59 U/ml. Besides another marker enzyme SGPT revealed the serum level of 40 U/ml. Similar results were obtained for triglycerides. The effects induced by hydrocortisone was compensated by the treatment with the extract of green tea as special diet. Studies pertaining to the effect of EGTE on hypersensitivity mediated ractions (IgE) in mice was measured by ELIZA which suggest EGTE an ingrediant from green tea extract might be useful in counter acting allergic responses. The study was further extended to determine hypertensive effect of green tea supplemented with egg led to a remarkable increase in total cholesterol. The increased activity of hepatic serum biomarkers SGOT and SGPT perhaps a well diagnostic indicators of hepatic injury.

Cite this article as: Soma Prabha, A., & Prabakaran, V., "Immunomodulation and Antihypertensity Study of Green Tea Extracts Induced with Hydrocortisone by Using Animal Model". *International Journal of Advanced Scientific Research & Development (IJASRD), 05* (05/I), 2018, pp. 01 – 12. https://doi.org/10.26836/ijasrd/2018/v5/i5/50501.

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INTRODUCTION

Green tea is the healthiest beverage on the planet. It is loaded with antioxidants and nutrients that have powerful effects on the body. This includes improved brain function, fat loss, a lower risk of cancer and many other incredible benefits. Green tea is more than just green liquid. Many of the bioactive compounds in the tea leaves do make it into the final drink, which contains large amounts of important nutrients. It is loaded with polyphenols like flavonoids and catechins, which function as powerful antioxidants. These substances can reduce the formation of free radicals in the body, protecting cells and molecules from damage. These free radicals are known to play a role in aging and all sorts of diseases. The chemical composition of green tea varies with climate, season, horticultural practices, and age of the leaf (Pastore Robert, 2005). The active constituents in green tea are powerful antioxidants called polyphenols. Tea is reported to contain nearly 4000 bioactive compounds of which one third is contributed by polyphenols (Tariq *et al.,* 2010).

Among the polyphenols in tea, is a family of compounds called the flavanoids. Flavanoids are the basic phenolic compounds in green tea responsible for antioxidant activities such as neutralization of free radicals that are formed in the process of metabolism (Horzic *et al.,* 2009). These flavanoids contains a substance called catechins. Major catechins present in green tea are epicatechin (EC), epigallocatechingalllate (EGCG), epigallocatechins (EGC) and epicatechingallate (ECG).

Antioxidants and other substances in green tea helps to protect the heart and blood vessels. The green tea, leaves are harvested, withered and then heated through steaming (Japanese style) or pan-firing (Chinese style) to produce green tea. This process halts oxidation so the leaves retain their color and delicate, fresh flavor. The caffeine content of green tea ranges from 24-40mg per cup and black tea ranges from 14-61mg per cup. The health promoting effects of green tea are mainly attributed to its polyphenol content (Naghma and Hasan, 2007), particularly flavanols, which represent 30% of fresh leaf dry weight. Green tea extracts are more stable than pure epigallocatechingallate, one of the major constituents of green tea, because of the presence of other antioxidant constituents in the extract (Osada *et al.,* 2001) Green tea helps in the prevention of certain types of cancers, fighting tooth decay and gum diseases. It also aids in weight loss.

Green tea and their constituents show a pivotal role in defense against pathogens through the modulation of immune system. Green tea consumption has been associated with increased bone mineral density and it has been identified as an independent factor protecting against the risk of hip fractures, (Muraki *et al.,* 2003). The present study Immunomodulation and Antihypertensity Study of Green Tea Extracts Induced with Hydrocortisone by Using Animal Model.

MATERIALS AND METHOD

2.1 Animals

Adult (90 ± 10 days) male albino rats of weighing 200 gm were chosen for their experiment. Animals were maintained as per National guidelines and protocols. Animals were reared in clean poly propylene cages and were maintained in a controlled environmental temperature (21-24°C) in an animal house and photoperiod of 12 hours of

light and 12 hours of darkness with free access to water. Animals were fed on standardized normal diet.

2.2 Preparation of Green Tea Leaf Extract

Aqueous extract of green tea leaf was prepared following the method of Wei *et al.,* (1999). Five gram of green tea was added to 100 ml of boiling water and was steeped for 15 min. The fusion was cooled to room temperature and was filtered. Tea leaves was extracted a second time with 100ml of boiling water and filtered. Two filtrates were then combined to obtain a 2.5% tea aqueous extract (2.5 gm tea leaves / 100 ml of water). The extract was then ready for oral administration.

2.3 Determination of Antimicrobial Susceptibility Test

The antibacterial activity of green tea extract obtained with distilled water was evaluated by the agar disc diffusion method. The 24 hours old culture was inoculated for the assay. Four bacterial pathogens are used for antimicrobial study. They are *Shigella flexneri, Pseudomonas aeruginosa, Pseudomonas fluorescence, Eschorichia coli*. A sterile colon swab was dipped into the bacterial suspension and then evenly swabbed over the entire surface of a sterile Muller Hinton agar plate to obtain uniform inoculums. The discs were punched on the seeded plates using a sterile cork borer and plates were allowed to dry for 5 minutes. The solvent extraction green tea extract were dispensed into each well using a sterile micro pipette. The cefotaxime acid disc was used as positive control. The plates were incubated for 24 hours at 37°C. The antibacterial activity was determined by measuring the diameter of zone on inhibition (mm).

2.4 Animal Treatment

Rats were equally divided into three groups (n=12). Initial body weights of all the rats were recorded.

- **Group I: Vehicle treated control group**

  A volume of distilled water equal to green tea extract was administered orally two times daily by intragastric gavage needle for five days, and physiological saline (0.9% NaCl) was administered intra peritoneal in similar dose of MTX after 72 hours from the beginning of the experiment.
- **Group II: Hydrocortisone induced treated group**

  The distilled water was administered orally two times daily for five days, and the animals received intra peritoneal injection of Hydrocortisone after 72 hour from the beginning of the experiment.
- **Group III: Green tea extract and Hydrocortisone induced group**

  2.5% aqueous green tea extract (1ml / 100gm) was administered orally two times daily by intragastric gavage needle for five days, and theanimal injected with hydrocortisone.

2.5 Evaluation of Biochemical Parameters

After completion of 26 days of treatment final body weights of all the rats were taken and the rats were anaesthetized one after another with chloroform and blood was collected directly from hepatic portal vein and allowed to coagulate, clear serum was collected and

stored in 20°C for enzyme assay. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalo-acetate transaminase (SGOT) were measured in all the control and experimental animals through the process of Kind and King, 2014. Finally results were compared with the respective controls. Triglycerides were determined by the method of Bucolo and David (1973) in an aqueous extract.

2.6 Haematological Parameters

Haematological parameters of polymorphic cells, lymphocyte cells, eosinophills were analyzed.

2.7 Immunomodulation Study – II

2.7.1 Animal

Female mice weighing 75g were obtained. The animals were housed in cages with ventilated micro isolator systems and were maintained at 21°C – 24°C on a animals received a standard sterile rodent chow diet and distilled water.

2.7.2 EGTE Administration

This study consisted the analyze innate immunity and adaptive immunity. In the first experiment, mice were randomly assigned to two groups.

- **Group I :** A vehicle control group administered distilled water (DW, 2ml / kg).
- **Group II:** A vehicle treated group, administered orally EGTE at 200mg / kg.

Distilled water and EGTE were daily administered for 2 weeks by oral gavage and then animals were then immunized and boosted with OVA albumin and the mice were then subjected to analysis of serum OVA-specific antibodies and splenocytes-expressed cytokines including IL-2, IFN-γ. Immunization with OVA significantly increased the serum levels of anti-OVA IgE compared with control mice. OVA-specific IgE were measured by ELISA.

2.8 Antihypertensive Activity

2.8.1 Egg Feed-Induced Hypertensive Model

Female albino mice weighing 150mg were divided into three groups.

- **Group – 1:** Animals in group one treated as control
- **Group – 2:** Animals in group two received orally a special prepared egg diet five egg yolk mixed with 50g of normal diet for fifteen days in order to produce cholesterol induced hypertension.
- **Group – 3:** Animals in group three received egg feed diet and aqueous extract of green tea (100mg / kg) for the same time period. All animals received distilled water instead of tap water. Blood pressure and heart rate of each of these groups were measured on week.

2.9 Biochemical Parameters

Biochemical parameters of Total Cholesterol, Triglycerides were measured.

2.10 Procedure for Catalase Activity

Erythrocyte sediment was prepared from the heparinized serum and washed 3 times with isotonic saline. A stock haemolysate containing approximately 5gHb/ dl was prepared.

By the addition of 4 parts by volume of distilled water at:500 dilution of this concentrated haemolysate with sodium-potassium phosphate buffer (0.05 ml, pH 7) was prepared immediately before the assay. Reference cuvette contained 1 ml of buffer and 2 ml of haemolysate and test cuvette contained 2 ml distilled haemolysate. The reaction was started by addition of 1 ml of H_2O_2 (30 mm in the buffer) to the test cuvette, mix well and the decrease in extinction was measured at 240 nm for 1minute by 15 sec interval.

RESULT

Green tea is widely consumed beverage known for its immunomodulatory effect, beneficial and anti-inflammatory, and antibacterial activity. In the present study Epigallocatechingallet fraction of green tea extracts were given to mice for four weeks and examined their effects on immune response by measuring phagocytic cells and antibody production. The mice were sacrificed two weeks after boosting for functional assessment of immune response and it is compared with control mice. The results noted on (Table: 1, Fig:1 and Plate: 1).

The HPLC elution profile of Catechin related compounds were identified. It was found out that administration of EGTE (Green tea) was found increase T cell B cell proliferation.

The ratio of proliferation with concanavallin as standard (5µg / ml). It is noteworthy to study the stimulation index. It is the ratio of proliferation with concanavallin divided by proliferation. The result suggested control mice showed 2.33 ± 0.22 with concanavallin. Whereas administration of EGTE 200 mg / kg exhibited 2.76 ± 0.22. Simultaneously with the concentration of EGTE increased to 400 mg / kg exhibited 3.11 ± 0.33. It was confirmed from the above study that, EGTE obtained from green tea extracts enhance the multiplication of leukocytes.

In vitro study showed that green tea extracts also increased the activity of serum such as expression of catalase enzymes which are implicated in cellular protection against reactive oxygen species (ROS). The results showed on (Table: 2 and Fig: 2).

The result suggested supplementation of green tea enhanced polymorphic cells observed as differential count expressed polymorphic cells of 42% cells / cubic mm / as hydrocortisone alone, mediated mice model, exhibited 40% of polymorphic cells. Similarly, on observation of lymphocyte cells which exhibited 58% cells / cubic mm with green tea and hydrocortisone combination. Whereas, when hydrocortisone alone was given it was found to be reduced to 55%. Similarly, the eosinophils compound count was found to be 03% cell /cubic mm when the mice were injected with green tea and hydrocortisone. Similar findings with eosinophils exhibited 02% which were found when it treated with hydrocortisone alone.

The effects of Serum Glutamic Pyruvic Transaminase and Serum Glutamic Oxaloacetic Transaminase as serum markers were measured for all the control and experimental animals. The impact of green tea on SGPT and SGOT activities were monitored for fifteen days with oral administration of green tea extracts in different group of animals. It was found out that the body weight was reduced after the treatment of green tea. On analysis of serum in mice model the activities of SGOT and SGPT as specific

markers in serum was found to increase is shown in the result exhibited on (Table: 3 and Fig: 3).

On treatment with hydrocortisone alone in mice model led to a significant immune suppression expressed to decrease in SGOT which exhibited 149 U / ml. Whereas, the green tea along with hydrocortisone analysis in the serum sample revealed serum glutamic oxaloacetic transaminase exhibiting an enhance level of 130 U / ml were estimated. The administration of green tea extracts significantly increased a serum level. Further study on evaluation on serum glutamic pyruvic transaminase analyzed in hydrocortisone treated mice which revealed 59 U/ml which is compared with another marker enzyme SGPT estimated by the method of Reitmann and Frankel, 1957 method which revealed a serum level of 40 U/ml. Administration of green tea significantly increased the level of serum SGPT (Plate: 2, 3, 4 and 5).

The study was further extended to elucidate the level of triglycerides in hydrocortisone induced mice. It was found to produce 56 mgs/dl. It is then compared with green tea supplemented with hydrocortisone treated mice model which exhibit a serum level of 75 mgs/dl. The effects induced by hydrocortisone were compensated by the treatment with the extract of green tea supplied as special diet. Rather compared to control which treated 60.0 for SGOT and 46.0 SGPT. Whereas, triglycerides level showed 42.5 mgs/dl.

Effect of EGTE on IgE response to OVA albumin to determine the effect of EGTE on adoptive immune response mice treated with EGTE for 15 days were immunized and boosted with OVA and mice then subjected to analysis of serum OVA specific antibodies. Immunization with OVA albumin increased the serum levels of anti-OVA IgE compared with serum from control mice. However anti OVA IgE were measured by ELISA and it was significantly increased compared to EGTE it suggested that EGTE might be useful in counter acting allergic responses. The results noted on (Table: 4 and Fig: 4).

IgE specific antibody production immunized with OVA-albumin treated with EGTE. The immunized mice control reveled IgE secretion with 0.16 ± 0.09 IU / ml of titer value. Whereas supplementation of green tea extract of 400mg / kg showed and IgE synthesis of 1.27 ± 0.05 U / ml titer value. The above result was in total agreement with study on Chao-Lin Kuo *et al.,* 2014. The presence study also supports catechin related molecule present in EGTE could be responsible for modulation of cytokine production and IgE secretion. Thus our study pertaining to supplementation of EGTE could have theoretical potential or application of treatment of allergic response (Plate: 6 and 7).

The present investigation determination of hypertension effects of green tea in mice model supplemented with egg indicator increased total cholesterol of 80.0mg / dl for egg fed hypertensive mice reveled increased serum total cholesterol compared to mice induced with green tea and egg albumin. A significant decrease with mice administered with green tea and fed with egg reveled a total serum cholesterol of 74.0 mg / dl. Similarly, determination of hypotensive activity in mice treated with egg alone reveled an increased content of serum triglycerides on 145mg / dl rather compared to other group of experiment with green tea and egg treated experiment reveled the serum triglycerides content of 126 mgs / dl was depicted in (Table: 5, Fig: 5 and Plate: 8). This study clearly envisaged reduction in serum triglycerides presumed to be the effect of green tea.

The aqueous extract of green tea was screened for their antibacterial activity against four gram negative bacterial pathogens such as *Shigella flexneri, Pseudomonas aeruginosa, Pseudomonas fluorescence and Escherichia coli.* The antibacterial activity of green tea extract was shown in Plate: 9. Cefotaxime (30µg) was used as reference standard for comparing the results. In the present investigation, the green tea extract was found to exhibit a maximum activity against *P. aeruginosa* with the inhibitory zone of 16mm, followed by *P. fluorescence* with 13mm. Similarly, the aqueous extract of green tea was found to be moderately active against *Escherichia coli* with 10mm and the minimum inhibitory activity was observed for *shigella flexneri* with the 9mm of zone of inhibition. The results revealed that, the aqueous extract of green tea was proved to have antibacterial effect against the multidrug resistant clinical pathogens with the work of Jazani et al, 2007 in which they emphasized that green tea was found to inhibit multidrug resistant *P. aeruginosa*. The antibacterial effect of green tea was mainly due to the presence of their polyphenolic components including epicatechin, epicatechingallate, epigallocatechin and epigallocatechingallate against gram negative bacteria (Zhao et al, 2002).

3.1 Haematological Analysis

Table – 1: *Multiplication of Cells from Mice Treated with Different Concentration of EGTE*

Sl. No.	Treatment Group	Concanavallin (std)
1	Control	2.33 ± 0.22
2	Expt – I (EGTE – 200ml)	2.76 ± 0.22
3	Expt – II (EGTE – 400ml)	3.11 ± 0.33

Table – 2: *Influence of Herbal Extract of Green Tea as Immunomodulator in Different Hepatic Cells*

Sl. No.	Treatment Group	Haematological Analysis		
		Polymorphic Cells	Lymphocyte Cells	Eosinophils
1	Control	38% cell / cubic mm	52% cells / cubic mm	1% cells / cubic mm
2	Expt – I (Hydrocortisone alone)	40% cells / cubic mm	55% cells / cubic mm	2% cells / cubic mm
3	Expt – II (Hydrocortisone + Extract)	42% cells / cubic mm	58% cells / cubic mm	3% cells / cubic mm

Table – 3: *Impact of Green Tea Extract on the Activities of Hepatic Serum Markers in Control and Experimental Treated Mice*

Sl. No.	Groups	SGOT U/L	SGPT U/L	Triglycerides
1	Control	60 U/ml	46 U/ml	42.5 mgs/dl
2	Expt – I (Hydrocortisone alone)	149 U/ml	59 U/ml	56 mgs/dl

3	Expt – II (Hydrocortisone + GTE)	130 U/ml	40 U/ml	75 mgs/dl

Table – 4: *Effect of Green Tea on Total Cholesterol and Triglycerides of Eff Feed Induced Hypertensive Mice*

Sl. No.	Treatment Group	Total Cholesterol	Triglycerides
1	Control	60.4 mg/dl	89 mg/dl
2	Egg Feed Alone	80.0 mg/dl	145 mg/dl
3	Egg Feed + GTE	74.0 mg/dl	126 mg/dl

Table – 5: *IGE Antigen-Specific Antibody Production in OVA-Immunized Mice Treated with EGTE*

Sl. No.	Treatment	Anti-OVA Antibody IGE (IU / ml)
1	OVA Immunized Mice Control	0.16 ± 0.09 IU/ml
2	EGTE 400 mg/kg	1.27 ± 0.05 IU/ml

FIGURE:-1

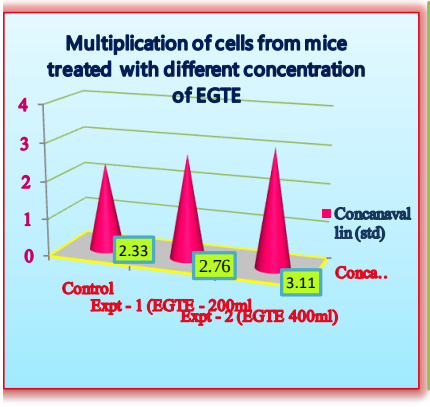


FIGURE:-2

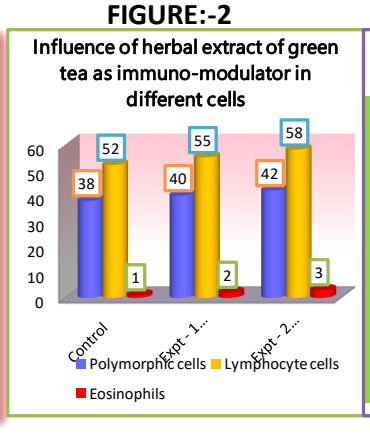


FIGURE:-3

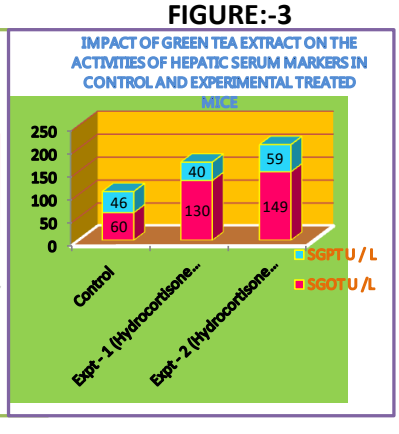


FIGURE:-3.1

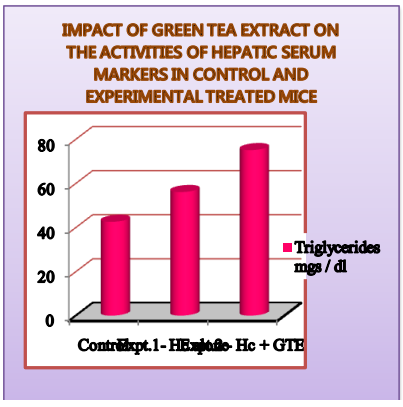


FIGURE:-4

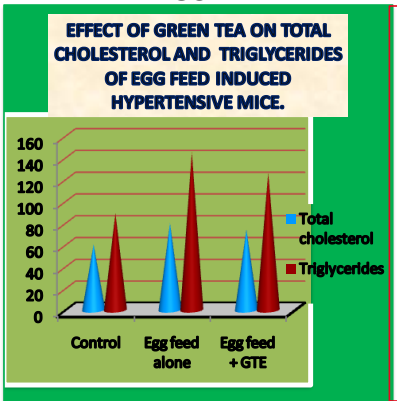


FIGURE:-5

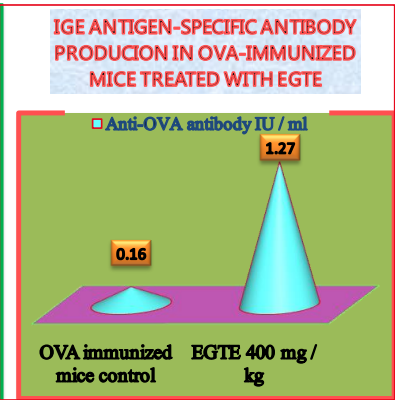


PLATE:1. PREPARATION OF AQUEOUS GREEN TEA EXTRACT

PLATE:2.MAINTENANCE OF EXPERIMENTAL

PLATE:3. ORAL ADMINISTRATION OF GREEN TEA EXTRACT GIVEN TO HYDROCORTISONE INDUCED MICE

PLATE:4. HYDROCORTISONE

PLATE – 5. INTRAPERITONEAL ADMINISTRATION OF HYDROCORTISONE TO EXPERIMENTAL MICE

PLATE:6.WEIGHING OF EXPERIMENTAL ANIMALS

PLATE.7..EXPERIMENTAL MICE ANAESTHETIZED WITH CHLOROFORM

PLATE – 8. BLOOD COLLECTION BY DIRECT HEART PUNCTURE

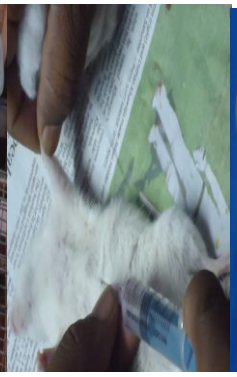
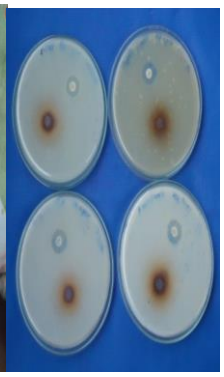


PLATE – 9.ANTIBACTERIAL ACTIVITY OF GREEN TEA EXTRACT AGAINST BACTERIAL PATHOGENS



DISCUSSION

Green tea is commonly consumed for its immunomodulatory effect. The immune modulatory properties of green tea are mediated through innate immune response. Tea extracts can also enhance interferon secretion and phagocytic activity, which increase the adaptive immune response. The epigallocatechingallet fraction of green tea extract given to mice for two weeks and examine the effects on innate and adaptive immune responses T polysaccharides is one the main components of green tea extract.

This catechin polysaccharides complex has been demonstrated to increase phagocytic activity which is an important component of immunomodulatory activity of green tea. The present study on haematological analysis was determined between the control treated group and the experimental groups. To examine the effects of EGTE with two different concentrations such as 200ml and 400ml were carried out. In order to study the effect of EGTE on innate and adaptive immune response experiment was carried out for two weeks at the end of the time mice were left unchallenged but immunized OVA - albumin simultaneously, the mice were sacrificed splenocyte were removed and stimulated with concanavallin 5mg / ml as std and the results were noted as proliferation assay from unimmunized or OVA – immunized mice, were performed. EGTE has increased T cell and B cell proliferation. Our results were in total conformity with the work of Chao-lin *et al.,* 2014. The concentration of EGTE green tea extract was increased significantly.

Influenced of herbal extract of green tea as immunomodulator in different groups of cells were monitored. Our data suggested hydrocortisone alone treated mice reveled 40% of polymorphic cells, 55% of lymphocyte cells and 0.2% of eosinophils cells whereas the hydrocortisone treated mice alone with green tea extract supplemented exhibited a slightly

enhanced level of polymorphic cells, lymphocytic cells and eosinophils. In the present study the effect of hydrocortisone could be shown by the reduction of total number of polymorphic, lymphocytic and eosinophilic cells. The immune suppression was induced by hydrocortisone. Our results were in total agreement with the study made by Bodinet *et al.,*2002 suggested that supplementation of herbal drugs which serve as immunomodulator in cytokines production. Besides when hydrocortisone and green tea extract was given to mice it cost a significant enhancement in polymorphic cells, lymphocytic cells and eosinophitic cells. Our data suggested that orally administer EGTE can significantly increased the ability of Nature killer cells. Leading to enhanced phagocytic activity by the innate immune system.

In another experiment performed with supplementation of green tea extract on the activities of hepatic serum markers SGOT, SGPT and Triglycerides were studied and the activities of hepatic serum markers SGOT, SGPT when treated with hydrocortisone alone showed decreased enzyme activity whereas, when hydrocortisone along with green tea extract reveled a significant increase of enzyme SGOT and SGPT.

The mice treated with hydrocortisone perhaps suppressed the immune responses and increased activity of serum SGOT, SGPT or well known diagnostic indicators of hepatic injury. In such cases liver damage with hepatocellular lesions enzyme were released from the liver into the blood stream. Besides SGOT and SGPT an important class of enzyme linking carbohydrate and amino acids cycle. Our results were in total agreement Vinoth Kumar *et al.,* 2010. In another study carried out presently with the activity total Triglycerides were in hydrocortisone treated mice will be decreased activity of triglycerides rather compared to control mice which reveled 42 mgs/dl which is worthy in to denote hydrocortisone supplemented with whereas hydrocortisone and green tea extract exhibited with moderate triglycerides activity with 56 mgs/dl. Thus present studies have shown hepato-productive effect of green tea is the highlight.

Despite the other factors, our data suggested a note worthy factor about modulation of cytokine production and IgE synthesis. The present study through carried out ELISA test. This presumes that induction of green tea extract maintained immune tolerance and regulate the production of allergic specific IgE.

The determination of hypertensive effects of green tea extract in mice model supplemented with egg and in another experiment mice were fed with egg albumin and white of yolk for fourteen days indicated an increased cholesterol level which led to an increased hypertension which may be presumed due to obesity. Simultaneously, the study was further modulated by supplementing green tea alone. There is also evidence that especially prepared egg feed which is rich in cholesterol also induced hypertension in rats (Saleem *et al.,* 2005). It is well reputed that one of the reason for glucose-induced hypertension is increase in sympathetic activity.

In the present investigation, the extract tested was found to significantly decrease the heart rate could be a strong reason of its antihypertensive effect in both normotensive and hypertensive rats. High cholesterol diet such as egg-feed diet is also associated with dyslipidemia as well as hypertension (Saleem *et al.,* 2005). In the present study the extract significantly reduced cholesterol level in rats which further justifies its antihypertensive effect. Endothelial dysfunction and oxidative stress are the important factors which lead to hypertension (Tomiyama *et al.,* 2000). Thus the antihypertensive effect of Menthalongifolia

could be due to the antioxidant effect of polyphenoles. Calcium channel blockers are an important family of drugs currently used in hypertension treatment. In a previous study it has been reported that Menthalongifolia have calcium channel blocking activity (Shah *et al.,* 2010), this mechanism can be a good explanation of its blood pressure lowering effect. The mice were found during the fourteen days of treatment with the extract. Similarly, biochemical parameters related to hepatic functions such as ALT, ASP and ALP were non – significantly reduced in rats at 500mg / kg when compared to control. AST is an enzyme found in the cytoplasm and mitochondria in different tissues, chiefly in the heart and skeletal muscles, liver, kidneys, pancreas, and erythrocytes (Aniagu *et al.,* 2004).

The reduction of these enzymes particularly AST and ALP indicated that the extract did not cause any toxic effects on skeletal muscles, liver, kidney, pancreas, and erythrocytes. In albino rats, the extract at doses of 500 and 100mg / kg demonstrated a significant decrease in total cholesterol, triglycerides, LDL levels while a prominent increase in HDL levels was observed. These effects are quite similar to lipid lowering drugs like stains (Barnett and Gara, 2003). This lipid lowering effect in rats could be due to its antioxidant effect.

CONCLUSION

Green tea is the healthiest beverage on the planet. It plays on important role in modulation of immune response, the antioxidant activity in green tea help to protect heart and vessels. The antimicrobial activity of green tea revealed a maximum zone of inhibition for *Pseudomonas aeruginosa* administration of EGTE 2mg/kg revealed 2.76 ± 0.22 it is to note green tea enhance multiplication of leukocytes, polymorphic cells mediated with hydrocortisone alone. Whereas green tea along with hydrocortisone found to be exhibit elevated level of leukocytes and polymorphic cells. The serum biomarker SGOT and SGPT found to be increase perhaps may be due to hepatic injury or liver damage with hapatogalaciens, further the total triglyceride were found to be decreased where hydrocortisone alone. Whereas and enhance activity were noted hydrocortisone and green tea. Thus presumes that induction of green tea maintained immune tolerance and regulate the production allergic specific IgE. Similar results were also observed in hypertensive mice model which is evidence by administering egg as feed rich in cholesterol induced hypertension. It is suggested that green tea is a safe beverage with active and powerful antioxidant called polyphenol utilized for neutralization of radicals.

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